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The overall goal of this research project is to investigate the cellular mechanisms associated with the heterosynaptic modulation of long-term synaptic potentiation (LTP) at mossy fiber synapses in hippocampus. It was previously shown that norepinephrine, through β-adrenoceptors, *enhances* the magnitude, duration, and probability of induction of mossy fiber LTP, while acetylcholine, through muscarinic receptors, *depresses* the magnitude and probability of induction of mossy fiber LTP. The goal for the first year of this research project was to test several specific hypotheses for the cholinergic and noradrenergic modulation of LTP. Specifically, the hypotheses relate to the possible requirement of postsynaptic calcium entry through voltage-gated calcium channels for the induction of LTP. We have also been investigating the properties of voltage-gated calcium channels in hippocampal CA3 neurons and the modulation of these calcium channels by noradrenergic and cholinergic agonists. These studies have used acutely exposed hippocampal neurons and a new preparation of isolated mossy fiber presynaptic terminals. In a collaborative project with Dr. David Terrian at the USAFSAM, San Antonio, the mechanisms of neurotransmitter release from a homogeneous fraction of mossy fiber synaptosomes have been investigated. Taken together, steady and significant progress has been made in a number of directions, all of which are associated with our attempt to understand mechanisms of excitatory synaptic transmission in the mammalian central nervous system and the neuromodulation of LTP by several neurotransmitters.

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Dr. William O. Berry	202-767-5021	NL

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HETEROSYNAPTIC MODULATION OF LONG-TERM POTENTIATION AT MOSSY FIBER SYNAPSES IN HIPPOCAMPUS

AFOSR 88-0142

Annual Technical Report

1 Summary

The overall goal of this research project is to investigate the cellular mechanisms associated with the heterosynaptic modulation of long-term synaptic potentiation (LTP) at mossy fiber synapses in hippocampus. It was previously shown that norepinephrine, through β-adrenoceptors, *enhances* the magnitude, duration, and probability of induction of mossy fiber LTP, while acetylcholine, through muscarinic receptors, *depresses* the magnitude and probability of induction of mossy fiber LTP. The goal for the first year of this research project was to test several specific hypotheses for the cholinergic and noradrenergic modulation of LTP. Specifically, the hypotheses relate to the possible requirement of postsynaptic calcium entry through voltage-gated calcium channels for the induction of LTP. We have also been investigating the properties of voltage-gated calcium channels in hippocampal CA3 neurons and the modulation of these calcium channels by noradrenergic and cholinergic agonists. These studies have used acutely exposed hippocampal neurons and a new preparation of isolated mossy fiber presynaptic terminals. In a collaborative project with Dr. David Terrian at the USAFSAM, San Antonio, the mechanisms of neurotransmitter release from a homogeneous fraction of mossy fiber synaptosomes have been investigated. Taken together, steady and significant progress has been made in a number of directions, all of which are associated with our attempt to understand mechanisms of excitatory synaptic transmission in the mammalian central nervous system and the neuromodulation of LTP by several neurotransmitters.

2 Research Objectives

The research objectives for the funding period 1 April 1988–31 March 1989 were as follows:

a) Test the hypothesis that a rise in intracellular calcium in postsynaptic CA3 neurons is required for the induction of mossy fiber LTP.

b) Test the hypothesis that the membrane potential of postsynaptic CA3 neurons is a variable for the induction of mossy fiber LTP.

c) Test the hypothesis that multiple types of voltage-gated calcium channels are present in the cell bodies and dendrites of CA3 pyramidal neurons.

d) Test the hypothesis that norepinephrine, through β-adrenergic agonists, enhances the activity of voltage-gated calcium channels in CA3 neurons.

e) Test the hypothesis that muscarinic cholinergic agonists depress voltage-gated calcium channels on CA3 pyramidal neurons.

f) Test the hypothesis that the N-type voltage-gated calcium channels are present at presynaptic mossy fiber terminals.

g) Test hypotheses for the mechanism of presynaptic autoregulation at the mossy fiber synapse.

h) Investigate the relative contribution of multiple types of presynaptic calcium channels to mossy fiber neurotransmitter release.

3 Status of Research

3.1 Test the hypothesis that a rise in intracellular calcium in postsynaptic CA3 neurons is required for the induction of mossy fiber LTP.

At most synapses in the hippocampus, activation of NMDA-type glutamate channels is required for the induction of LTP (1). It has been hypothesized that calcium influx through these NMDA channels is the requisite first step in the induction of LTP (8, 9). In contrast, LTP at mossy fiber synapses is *independent* of the activation of NMDA receptors (5), and there is a very low density of NMDA receptors in the vicinity of these synapses (11). It is obvious, therefore, that some other mechanism must be involved in the induction of mossy fiber LTP. We have proposed, based on previous work, that activation of postsynaptic voltage-gated calcium channels by the high frequency stimulation normally used to induce LTP, and the subsequent calcium entry through these calcium channels, provides an alternate, yet analogous, mechanism for mossy fiber LTP (7). One prediction of this hypothesis is that chelation of postsynaptic calcium should block the induction of mossy fiber LTP. Experiments were performed using intracellular injection of two calcium chelators, BAPTA and QUIN-2. The preliminary results of these experiments appear to support the hypothesis, that is, cells into which BAPTA or QUIN-2 was injected displayed significantly less LTP than a control group of cells without the injection of calcium chelators.

3.2 Test the hypothesis that the membrane potential of postsynaptic CA3 neurons is a variable for the induction of mossy fiber LTP.

The second prediction of the hypotheses outlined in the preceding section is that the membrane potential of the postsynaptic neuron during high frequency stimulation of the mossy fibers should be a variable for the induction of LTP. In other words, hyperpolarization below threshold for activation of voltage-gated calcium channels during high frequency stimulation should block the induction of LTP. Furthermore, depolarization during high frequency stimulation should enhance the magnitude and probability of induction of LTP. The results of the preliminary experiments appear to support this hypothesis. We found that hyperpolarization blocked mossy fiber LTP, and depolarization enhanced LTP. These experiments are continuing and will be discussed further in next year's technical report.

3.3 Test the hypothesis that multiple types of voltage-gated calcium channels are present in the cell bodies and dendrites of CA3 pyramidal neurons.

It has been reported in a number of preparations that at least three types of voltage-gated calcium channels exist on neurons (10). In our studies of voltage-gated calcium channels in granule cells, we

obtained preliminary evidence that there were three types (T, N, and L) (4). We wanted to test the hypothesis that these three types of calcium channels were present in CA3 pyramidal neurons and to explore the distinguishing characteristics of the three types of channels. Figures 1–3 illustrate the differences in the three types of calcium channels observed in CA3 cells. The T channel has the smallest single channel conductance, inactivates rapidly with depolarization, and has the lowest threshold for activation. The L channel, in turn, has the largest single channel conductance, inactivates relatively little with prolonged depolarization, and has the highest threshold for activation. The N channel has characteristics that fall in the middle of the T and L.

3.4 Test the hypothesis that norepinephrine, through β-adrenergic agonists, enhances the activity of voltage-gated calcium channels in CA3 neurons.

With the characterization of at least three types of calcium channels in CA3 pyramidal neurons, it became feasible to test the hypothesis that norepinephrine, through β-adrenoceptors, modulates specific types of calcium channels. Briefly, we found that isoproterenol enhanced the activity of the N- and the L-type channels with essentially no effect on the T-type channel. This finding begins to narrow the range of possibilities for the involvement of calcium channels in mossy fiber LTP. If the enhancement of LTP by β-adrenergic agonists is through enhancement of calcium channel activity, then the N-type, the L-type, or both must be involved, but not the T-type channel.

3.5 Test the hypothesis that cholinergic muscarinic agonists depress voltage-gated calcium channels on CA3 pyramidal neurons.

An obvious hypothesis that we derived from our finding that muscarine depressed mossy fiber LTP is that muscarinic agonists might depress voltage-gated calcium channels. We have investigated the effects of carbachol on three types of voltage-gated calcium channels. The results are highly preliminary and appear to be somewhat complex. Thus far, we have found that carbachol depresses the L-type calcium channel. However, carbachol appears to increase the T-type channel and may also increase the N-type channel. If these initial observations are confirmed with further experiments, then, taken together with the results outlined above, one would have to conclude that the L-type channel must be involved in the induction of LTP. We are very excited about these preliminary results and will be pursuing them aggressively during the next funding period.

3.6 Test the hypothesis that the N-type voltage-gated calcium channels are present presynaptically in mossy fiber synaptic terminals.

Based on indirect evidence, it has been hypothesized by others that the N-type calcium channel is involved in transmitter release at nerve terminals (6). With our ability to record directly single calcium channels from isolated mossy fiber synapses, we felt it was extremely important to test this hypothesis. Progress in our investigation of voltage-gated calcium channels at presynaptic mossy fiber terminals has been slow but steady. We are progressively accumulating more and more recordings of single calcium channels from isolated presynaptic terminals. Figure 4 illustrates the results of our experiments to date. We have measured calcium channels with single channel conductances ranging from about 2–25 picosiemens. The classification of the channel type has been very difficult, especially compared to the relatively easy classification of calcium channels recorded from the cell bodies and dendrites of the same neurons. We are tentatively concluding that the L type channel is not present at the mossy fiber terminal based on the insensitivity of the channels to BAY K 8644. Beyond that conclusion, however, we become quite tentative. There appears to be quite a heterogeneous mixture of channel types at presynaptic terminals, and we are as yet unsure

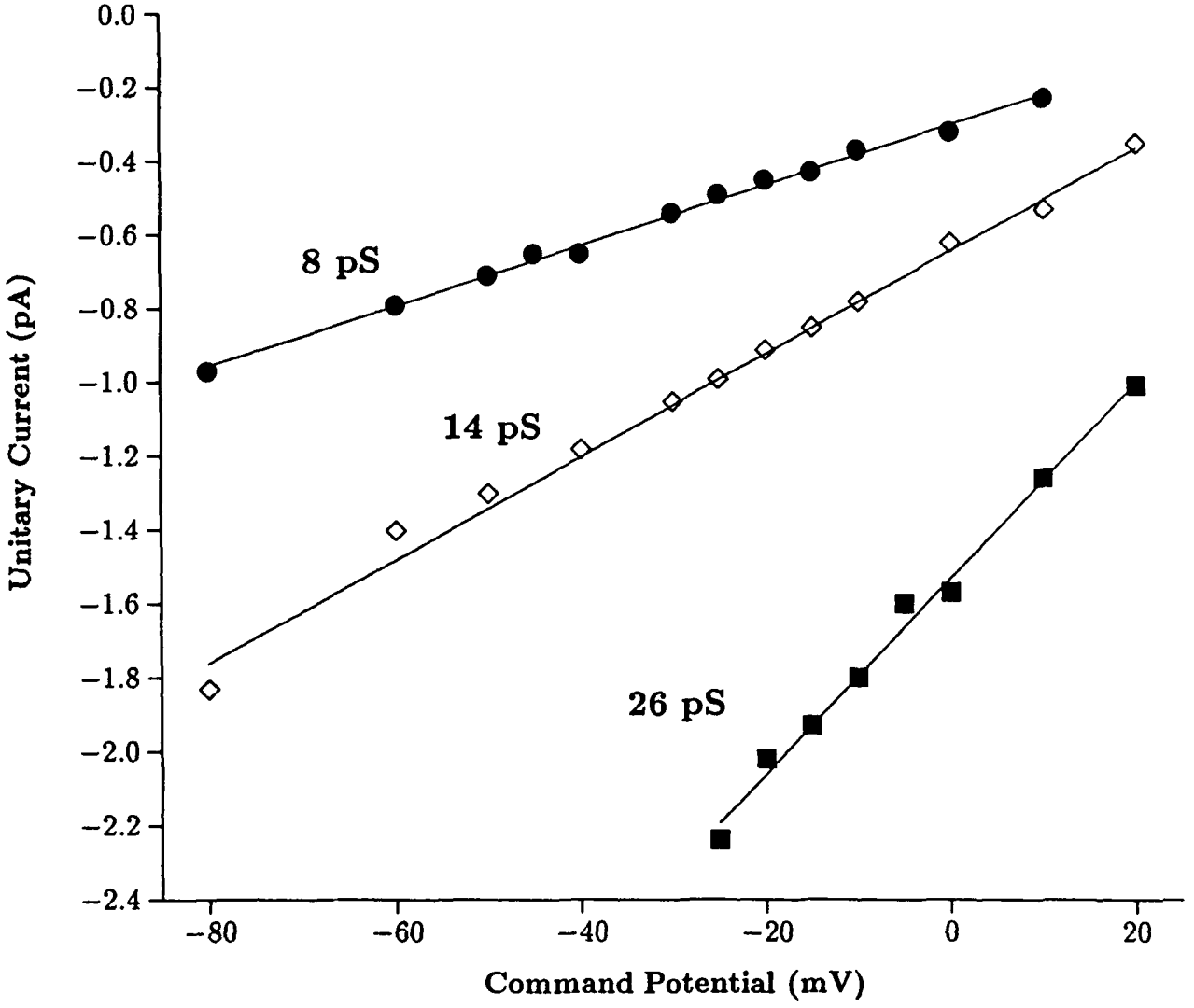

Figure 1: The three classes of channels have distinct single channel conductances.
Top line: data averaged from 13 patches containing the T-type channel.
Middle line: data averaged from 14 patches containing the N-type channel.
Bottom line: data averaged from 7 patches containing the L-type channel. Lines were fit by linear regression.

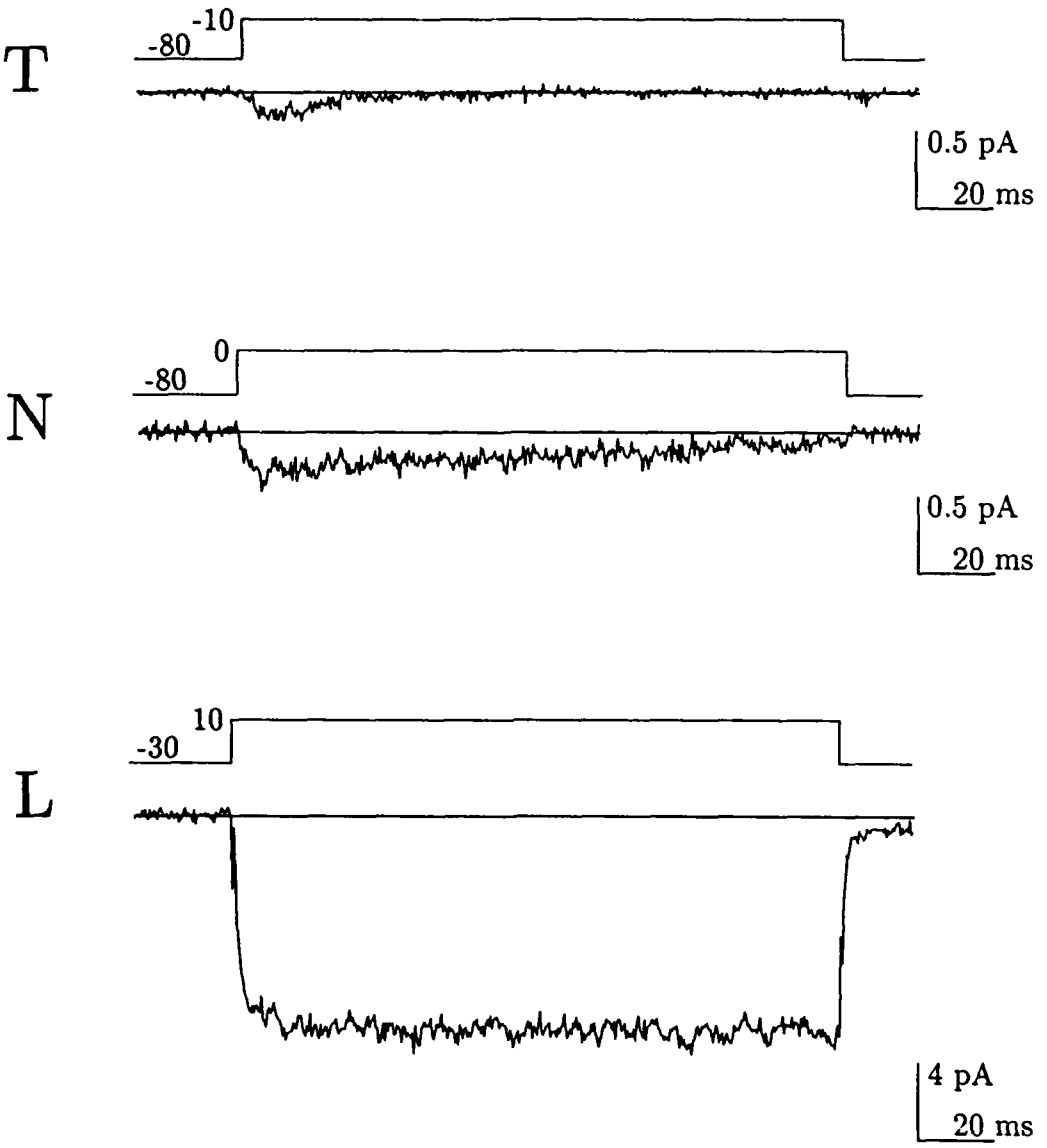

Figure 2: Ensemble averages from three different patches. The top trace is from a patch containing only T-type channels and shows complete channel inactivation during the command step (average of 226 traces). The middle patch contained only N-type channels and shows significant inactivation during the command (average of 40 traces). The bottom patch contained predominantly L-type channels and shows no significant inactivation during the command step (average of 44 traces).

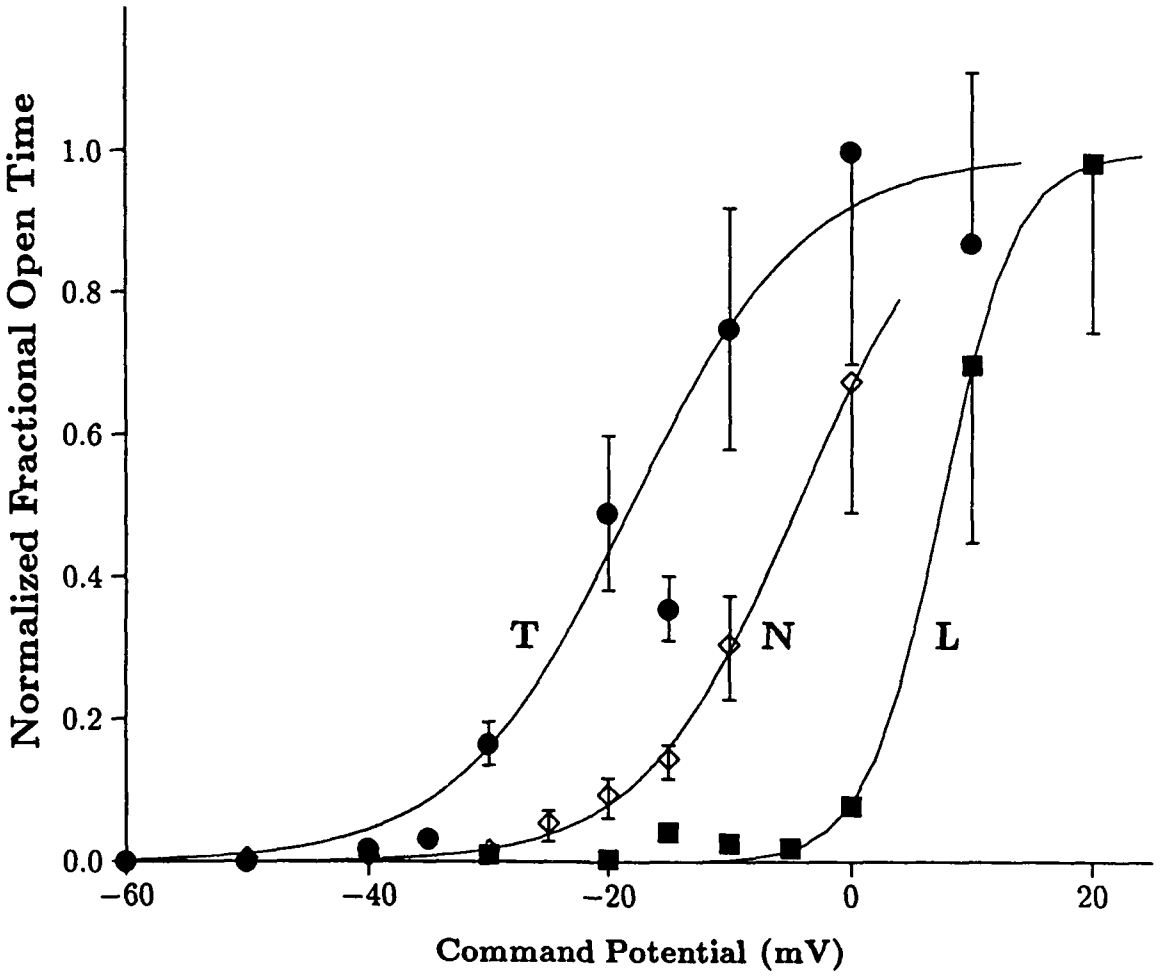

Figure 3: The three channel types show differences in voltage range of activation. Activation curves for T, N, and L channels derived from single channel records. For the T and N curves, the patches were held at -80 mV and stepped to the indicated command potentials. For the L curve, patches were held at -40 mV. At all potentials, deviations from the baseline of greater than 0.6 times the unitary current amplitude were considered as representing the open state. Because of inactivation, only data points between 2 and 30 ms after the command step were analysed in patches containing T channels. Half-activation points for each of the curves are: −18 mV (T), −4 mV (N), and 7 mV (L). Each curve is the average of several experiments: 10 (T), 8 (N), and 4 (L).

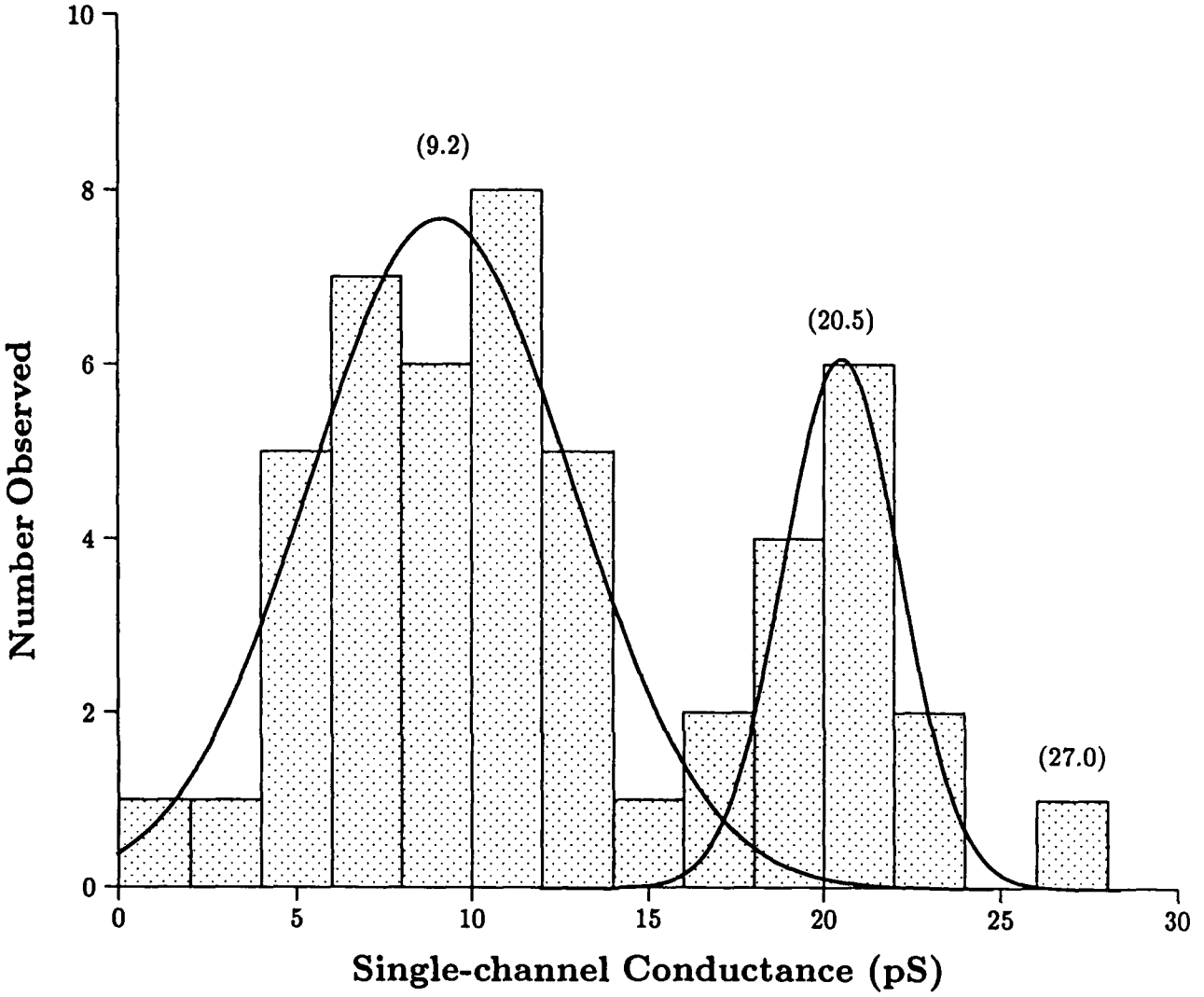

Figure 4: Distribution of single channel slope conductances calculated from single channel recordings in isolated mossy fiber terminals. The solid curves are the individual Gaussian distributions that best fit the data. Mean values are indicated above each peak. Binwidth was 2 pS.

whether they represent novel classes of calcium channels from those observed in cell bodies. These experiments will be pursued intensely in the next funding period.

3.7 Test hypotheses for the mechanism of presynaptic autoregulation at the mossy fiber synapse.

During this reporting period, we published the details of a new method developed by Dr. David Terrian for isolating intact hippocampal mossy fiber synaptosomes (13). The metabolic and morphological characteristics of this synaptosomal preparation were also described. We have since made use of this new preparation for testing three hypotheses concerning the autoregulation of neurotransmitter release from mossy fiber terminals that were based on previous electrophysiological studies conducted in this and several other laboratories.

a) The first hypothesis was that glutamate, acting at L (+)-2-amino-4-phosphonobutyrate (APB)-sensitive receptors, decreases the stimulated release of both endogenous glutamate and prodynorphin-derived peptides from mossy fiber synaptosomes. It had previously been demonstrated that APB potently blocks evoked excitatory postsynaptic potentials at the mossy fiber synapse without altering either the membrane potential or input resistance of the postsynaptic membrane.

b) The second hypothesis was that endogenous adenosine depresses the release of glutamate and dynorphin (Dyn) peptides. Electrophysiological studies had previously been able to provide indirect support for such a presynaptic action of adenosine, but no direct biochemical evidence was available.

c) The third hypothesis was that unesterified free fatty acids, liberated from membrane phospholipids in response to depolarization-induced calcium entry, selectively stimulate the release of cytosolic glutamate by modulating (Na^+, K^+) ATPase activity in the plasma membrane of mossy fiber terminals. This hypothesis was based on the work conducted previously by Dr. Terrian on the possible role of arachidonic acid in the release of amino acid neurotransmitters.

The results of the experiments that were conducted during this past year to test these hypotheses have either been published (2) or are currently in press (3, 12) and can be summarized as follows:

a) Our biochemical studies confirmed that APB acts at a presynaptic site to inhibit the evoked release of both glutamate and Dyn peptides from guinea pig but not rat mossy fiber synaptosomes. Although the mechanism for this presynaptic inhibition remains uncertain, our results were consistent with the suggestion that APB selectively inhibits the calcium-dependent component of glutamate release without affecting the release of cytosolic glutamate.

b) The results of these studies demonstrated that endogenous ATP is released by depolarized mossy fiber synaptosomes and rapidly hydrolyzed to adenosine. The extracellular adenosine derived from this source acts to inhibit the calcium-dependent release of glutamate and Dyn peptides. ATP itself did not appear to account for this presynaptic inhibition.

c) It was observed that membrane depolarization increases the accumulation of unesterified free arachidonate by a calcium-dependent mechanism and that this free fatty acid stimulates the release of endogenous glutamate

3.8 Investigate the relative contribution of multiple types of presynaptic calcium channels to mossy fiber neurotransmitter release.

The release of biogenic amine neurotransmitters has been shown to be potently inhibited by N-type calcium channel blockers, while calcium entry via L-type channels appears to be required for the release of neuropeptides from the rat neurohypophysis. Based on this information, we hypothesized that the dihydropyridine derivatives (L-type channel blockers) might selectively inhibit the evoked release of Dyn but not glutamate from mossy fiber synaptosomes. However, our results indicate that the presynaptic N-type calcium channels make the most substantial contribution to the calcium influx required for the exocytosis of prodynorphin-derived peptides from these isolated nerve endings. The release of Dyn was found to be relatively insensitive to dihydropyridines, suggesting that L-type calcium channels play little, if any, role in the release of these opioids in the mammalian hippocampus.

4 Publications

4.1 Full papers and review articles

1. Terrian, D.M., Johnston, D., Claiborne, B.J., Ansah-Yiadom, R., Strittmatter, W.J., and Rea, M.A. Glutamate and dynorphin release from a subcellular fraction enriched in hippocampal mossy fiber synaptosomes. *Brain Res. Bull.* 21:343-351, 1988.

2. Williams, S.H. and Johnston, D. Muscarinic depression of an APV-insensitive form of LTP in hippocampal CA3 neurons. *Science* 242:84-87, 1988.

3. Gray, R., Fisher, R., Spruston, N., and Johnston, D. Acutely exposed hippocampal neurons: A preparation for patch clamping neurons from adult hippocampal slices. In: In Vitro *Preparations From Vertebrate Nervous Systems.* Jahnsen, H. (ed.), John Wiley: England, (in press).

4. Hopkins, W.F. and Johnston, D. Noradrenergic modulation of synaptic plasticity in the hippocampus. In: *Developmental Neurophysiology.* Kellaway, P. and Purpura, D.P., (eds.), Johns Hopkins Univ. Press: Baltimore, (in press).

5. Johnston, D., Hopkins, W.F., and Gray, R. The role of norepinephrine in long-term potentiation at mossy fiber synapses in the hippocampus. In: *Neural Models of Plasticity.* Byrne, J.H. and Berry, W.O. (eds.), Academic Press, Inc.: San Diego (in press).

6. Johnston, D., Williams, S.H., Gray, R, and Fisher, R.E. Cholinergic and noradrenergic modulation of long-term potentiation in hippocampal CA3 neurons. In: *Brain Signal Transduction and Memory.* Ito, M. and Nishizuka, Y. (eds.), Academic Press, Inc.: San Diego, 1989, (in press).

7. Jaffe, D. and Johnston, D. Voltage-dependence of long-term potentiation at hippocampal mossy fiber synapses. (submitted)

8. Williams, S. and Johnston, D. Long-term potentiation of hippocampal mossy fiber synapses is blocked by postsynaptic injection of calcium chelators. (submitted)

4.2 Abstracts

1. Johnston, D. and Williams, S.H. Muscarine depresses long-term potentiation in CA3 neurones of the rat hippocampus. *J. Physiol.* 407:49P, 1988.

2. Fisher, R.E., Gray, R., and Johnston, D. β-adrenoceptor modulation of calcium channels in acutely exposed CA3 pyramidal neurons of adult guinea pig hippocampus. *Soc. Neurosci. Abstr.* 14:645, 1988.

3. Gray, R. and Johnston, D. Recordings of single calcium channels from presynaptic mossy fiber terminals in adult guinea pig hippocampus. *Soc. Neurosci. Abstr.* 14:68, 1988.

4. Jaffe, D.B. and Johnston, D. Depression of synaptic transmission by ω-conotoxin in the rat hippocampal slice. *Soc. Neurosci. Abstr.* 14:810, 1988.

5. Williams, S.H. and Johnston, D. Muscarine depresses an APV-insensitive form of LTP in CA3 hippocampal neurons. *Soc. Neurosci Abstr.* 14:564, 1988.

6. Williams, S.H. and Johnston, D. Hippocampal CA3 mossy fiber LTP is blocked by postsynaptic calcium chelators. *Soc. Neurosci. Abstr.* (in press), 1989.

7. Jaffe, D.B. and Johnston, D. Voltage-dependence of LTP at the hippocampal mossy fiber synapse. *Soc. Neurosci. Abstr.* (in press), 1989.

5 Professional Personnel Associated With the Research Project

Daniel Johnston, Ph.D.—Principal Investigator
Richard A. Gray, Ph.D.—Postdoctoral Fellow
Ron Fisher—Graduate Student
David Jaffe—Graduate Student
Nelson Spruston—Graduate Student
Mahmud Haque—Computer Systems Manager
Judy Walker, M.S.—Research Technician
David Terrian—Co-investigator
Anna Marie Michel—Research Associate

6 Interactions

04/16/88– 04/21/88	Spring Hippocampal Research Conference, St. Thomas
04/16/88– 04/21/88	Mahmud Haque to Masscomp Users' Society Meeting in Boston
05/15/88– 05/17/88	Association of Neuroscience Departments and Programs Spring Meeting and Congressional Visits
06/01/88– 06/03/88	NIMH Study Section, Washington, DC
07/19/88	Spoke to students in Baylor's SMART Program
08/18/88	To Department of Physiology, University of Texas, Dallas
09/20/88	Gave seminar in Physiology Department, BCM
10/03/88	Gave lecture in Department of Physiology, UT, Dallas
10/12/88– 10/14/88	NIMH Study Section, Washington, DC
11/12/88– 11/16/88	Society for Neuroscience, Toronto, Canada
11/27/88– 11/30/88	5th Takeda Science Foundation on Bioscience, Kyoto, Japan
01/21/89– 01/26/89	Winter Conference on Brain Research, Snowbird, UT
02/22/89– 02/23/89	NIA site visit, Rockville, MD
03/06/89– 03/08/89	NIMH site visit, Portland, Oregon
03/29/89– 03/30/89	Gave lecture in Department of Biology, Brandeis University

7 New Discoveries, Inventions, or Patent Applications

None.

8 References

1. COLLINGRIDGE, G.L., KEHL, S.J. AND MCLENNAN, H. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *J. Physiol. (Lond.)* 334: 33–46, 1983.

2. DORMAN, R.V., SCHWARTZ, M.A. AND TERRIAN, D.M. Depolarization-induced [^{3}H] arachidonic acid accumulation: effects of external Ca^{2+} and phospholipase inhibitors. *Brain Res. Bull.* 21: 445–450, 1988.
3. GANNON, R.L., BATY, L.T. AND TERRIAN, D.M., L(+)-2-amino-4-phosphonobutyrate inhibits the release of both glutamate and dynorphin from guinea pig but not rat hippocampal mossy fiber synaptosomes., *Brain Res. (in press).*
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11. MONAGHAN, D.T. AND COTMAN, C.W. Distribution of *N*-methyl-D-aspartate-sensitive L-[^{3}H]glutamate-binding sites in rat brain. *J. Neurosci.* 5: 2909–2919, 1985.
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13. TERRIAN, D.M., JOHNSTON, D., CLAIBORNE, B.J., ANSAH-YIADOM, R., STRITTMATTER, W.J. AND REA, M.A. Glutamate and dynorphin release from a subcellular fraction enriched in hippocampal mossy fiber synaptosomes. *Brain Res. Bull.* 21: 343–351, 1988.